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Research Note

VIBRATIONAL SPECTRA OF METHYL- d_3 L-ALANINES AND THE ASSIGNMENT OF THE C-H VIBRATIONS IN L-ALANINE

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Abstract—The i.r. and Raman spectra of polycrystalline methyl deuterated L-alanine and its N-deuterated derivative have been obtained and most of the fundamental frequencies have been assigned. Based on these data, revised assignments for the C-H stretching and deformation modes of normal alanine are proposed.

INTRODUCTION

The vibrational spectra of L-alanine and several deuterated analogs have been extensively studied and assignments for the fundamental frequencies have been proposed [1-7]. The assignments of a number of fundamentals, including those for the C-H stretching and deformation vibrations, nevertheless, remain uncertain. We have now investigated the i.r. and Raman spectra of the previously unreported isotopomer, $CD_3CH(NH_3^+)COO^-$, and its N-deuterated analog, and assigned their fundamental vibrations. Based on the observed number and positions of bands associated with the vibrations of the undeuterated methyne ($C^{\alpha}H$) group for these species, we propose a revised interpretation of the C-H stretching and deformation modes in the normal molecule. These results should prove helpful in assigning the C-H vibrations in other amino acids and should also provide a better understanding of the vibrational circular dichroism spectra for L-alanine [5] and other structurally similar, optically active species of biological importance.

EXPERIMENTAL

L-alanine- CD_3 (having an isotopic purity of $>98\%$) was purchased from Merck, Sharp and Dohme, Canada, Ltd.* and used without further purification. Samples for the i.r. spectra of this molecule were prepared as CsI pellets and as mulls between CsI plates. The N-deuterated derivative was prepared from the methyl- d_3 species by recrystallization from D_2O *in vacuo*. Infrared spectra for this species were obtained of Halocarbon and Nujol mulls. (CsI pellets proved impractical because of the ease with which the amino group undergoes isotopic exchange with trace amounts of water at high pressure). A Perkin-Elmer model 467 instrument, capable of scanning from $4000-250\text{ cm}^{-1}$, was employed. Raman spectra of the polycrystalline solids sealed in a glass melting point capillary were taken from 40 to 3300 cm^{-1} on a Spex 1401 spectrometer using the 514.5 nm line of a Spectra-Physics Model 165-03 laser. The laser power was $\sim 250\text{ mW}$ at the sample; the bandpass was $<3\text{ cm}^{-1}$. All spectra were taken at ambient temperature.

RESULTS AND DISCUSSION

Figure 1 presents the i.r. and Raman spectra of methyl deuterated alanine, while Table 1 summarizes the proposed vibrational assignments. The spectra and assignments for L-alanine- d_6 are presented in Fig. 2 and Table 2, respectively. Aside from the C-H stretches and deformations discussed below, these assignments were ar-

rived at by comparison with the spectra of previously investigated isotopic analogs [1-7]. Figure 3 presents a schematic correlation of the vibrational frequencies in the CH stretching and deformation regions of $CD_3CH(NH_3^+)COO^-$ and of normal L-alanine. Only very small shifts in the CH stretching and deformation frequencies are observed upon N-deuteration of these two molecules. Thus, the inferences and conclusions drawn from this figure and discussed in the text below for L-alanine and its methyl deuterated derivative, apply equally well to the N-deuterated isotopomers.

$3100-2800\text{ cm}^{-1}$

Only one intense band* at 2969 cm^{-1} appears in the Raman spectrum of the methyl deuterated analog in the CH stretching region. Obviously, it must be due to the stretching mode of the methyne group, $\nu(C^{\alpha}H)$. Of the five medium-to-strong high frequency Raman bands observed for normal alanine [3], the band at 2967 cm^{-1} must correspond to the same vibration. N-deuteration has little effect on the position of the $C^{\alpha}H$ stretching mode, and apparently deuteration of the methyl group causes no appreciable frequency shift either.

By comparison with well-known methyl group frequencies [8], three other Raman bands at 3000 , 2986 and 2932 cm^{-1} [3] are then easily attributed to the three CH_3 stretching modes as indicated in Fig. 3. The remaining weak Raman band at 2890 cm^{-1} in the spectrum of normal alanine [3] is most likely the overtone $2\delta_a(CH_3)$ in Fermi resonance with the symmetric CH_3 stretch at 2932 cm^{-1} . The CH stretching bands are not observed in the i.r. spectra of either normal alanine or its methyl deuterated isotopomer because of the overlap with exceedingly strong and broad NH stretching modes, as well as numerous overtones and combination bands. The very weak CH stretching bands, however, become visible in the infrared upon N-deuteration (Fig. 2 and Table 2).

$1500-1250\text{ cm}^{-1}$

The isotopically unsubstituted compound has two bands at 1482 and 1462 cm^{-1} in the solid state spectra. Both bands are missing in the methyl deuterated molecules. Thus the previously suggested assignment of these features to the asymmetric CH_3 deformation modes in the normal molecule is certainly correct [1-4]. The 20 cm^{-1} splitting, which is not observed for spectra of aqueous solutions [1], has been reported by Machida *et al.* [7] to arise from factor group interactions and not from the loss of degeneracy as is often found for a low symmetry molecule.

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

* Other Raman features seen here have quite low intensities as expected for the weakly scattering NH stretching modes or for overtones and combination bands (Table 1).

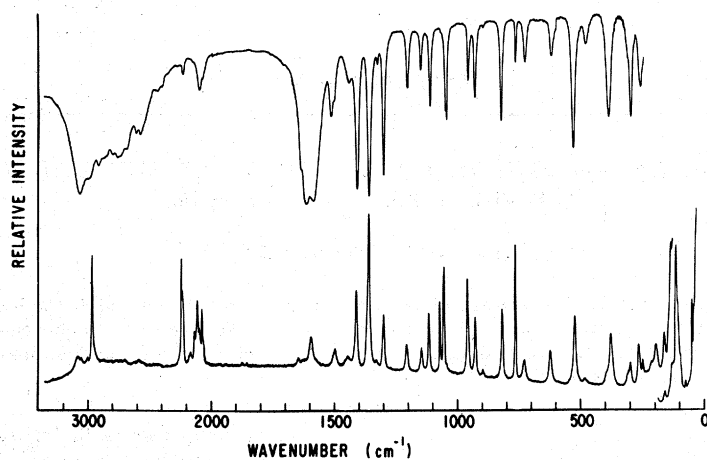
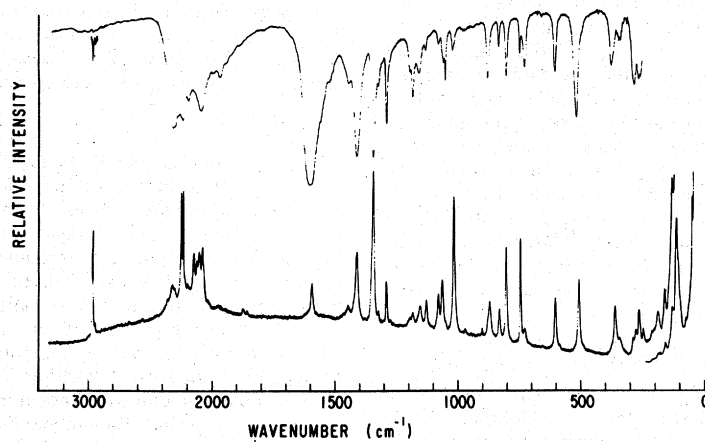
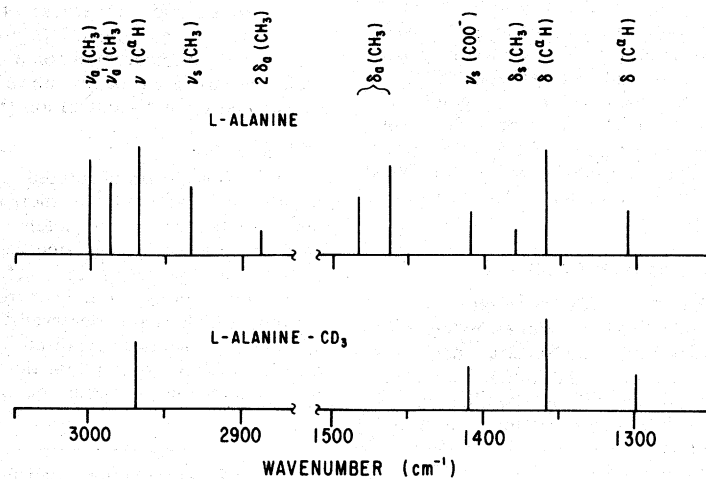
Fig. 1. Infrared and Raman spectra of polycrystalline L-alanine-methyl- d_3 .Fig. 2. Infrared and Raman spectra of polycrystalline L-alanine- d_6 .Fig. 3. A correlation diagram of Raman lines in the C-H stretching and deformation regions of L-alanine- d_0 and L-alanine-methyl- d_3 . (Frequencies for the d_0 species are from reference 3).

Table 1. Observed vibrational bands and assignments for crystalline L-alanine- d_3

Raman (cm^{-1})		i.r.* (cm^{-1})	Assignment†
~3084	(1)	3080 vs, br	$\nu_a(\text{NH}_3^+)$
3050 sh	(0)		
3001	(0)	2990 sh	$\nu_s(\text{NH}_3^+)$
2969	(7)		$\nu(\text{C}^\alpha\text{H})$
		(*)	
2245	(7)	2239 vvw	$\nu_a(\text{CD}_3)$
2236	(5)	2233 vvw	
2230 sh	(3)		
2171	(1)		
2142	(2)		
2115	(5)		
2100 br	(2)	2101 vw	
2078	(4)	2074 vvw	$\nu_s(\text{CD}_3)$
~2063	(1)		
~1648	(0)	1639 sh	$\delta_a(\text{NH}_3^+)$
~1623	(0)	1619 vs	
1598	(2)	1583 vs	$\nu_a(\text{COO}^-)$
		1554 sh	
		1513 mw	
1498	(1)	1502 w	$\delta_s(\text{NH}_3^+)$
1445	(0)	1434 w	
1410	(5)	1409 s	$\nu_s(\text{COO}^-)$
1358	(10)	1359 s	$\delta(\text{C}^\alpha\text{H})$
1327	(0)	1326 vvw	$\delta'(\text{C}^\alpha\text{H})$
1299	(4)	1301 s	
1207	(2)	1203 mw	$\left\{ \begin{array}{l} \rho(\text{NH}_3^+), \rho'(\text{NH}_3^+), \\ \text{and } \nu \text{ (skeletal)} \end{array} \right\}$
1146	(1)	1149 w	
1116	(4)	1110 mw	
1070	(6)		$\delta_s(\text{CD}_3)$
1053	(7)	1048 mw	$\delta_a(\text{CD}_3)$
		1043 m	
958	(7)	955 w	$\left\{ \begin{array}{l} \text{two } \nu \text{ (skeletal),} \\ \text{two } \rho \text{ (CD}_3\text{),} \\ \text{and } \gamma \text{ (COO}^-) \end{array} \right\}$
928	(4)	928 mw	
		894 vvw	
		820 m	
817	(5)	820 m	
763	(8)	762 vw	
726	(1)	723 vw	
		667 vvw	
619	(2)	618 vw	$\delta(\text{COO}^-)$
520	(6)	529 ms	$\rho(\text{COO}^-)$
478 br	(1)	~478 vw	$\tau(\text{NH}_3^+)$
~392	(1)	~386 m	$\delta(\text{C}^\beta\text{C}^\alpha\text{N})$
374	(4)		
308 sh	(1)		
297	(2)	296 m	two δ (skeletal)
263	(3)	256 mw	
246	(2)		
~220	(1)		
191	(2)		$\left\{ \begin{array}{l} \tau(\text{CD}_3), \tau(\text{COO}^-), \\ \text{and lattice modes} \end{array} \right\}$
157	(2)		
131	(4)		
112	(25)		
104 sh	(5)		lattice modes
96 sh	(4)		
73 sh	(2)		
47	(13)		

* The i.r. spectrum exhibits a number of overlapping overtone and combination bands between 2950–2300 cm^{-1} : 2920, 2850 sh, 2815 sh, 2773, 2743, 2688, 2621, 2587, 2435, 2398, 2345, 2322 cm^{-1} .

† Except for bands due to $\nu(\text{CD}_3)$, $\delta(\text{CD}_3)$, $\nu(\text{C}^\alpha\text{H})$, $\delta(\text{C}^\alpha\text{H})$, and $\nu(\text{NH}_3^+)$, the assignments for this molecule are analogous to those given in earlier references [1–4, 6]. Because strong coupling is likely between the $-\text{CD}_3$ and $-\text{NH}_3^+$ rocking modes and the skeletal stretching vibrations, specific assignments of group frequencies do not seem possible for these vibrations.

Table 2. Observed vibrational bands and assignments for crystalline L-alanine- d_6

Raman (cm^{-1})		i.r. (cm^{-1})	Assignment†
3001	(0)		
2972	(8)	2969 vw	$\nu(\text{C}^\alpha\text{H})$
2950	(1)		
2359 sh	(1)	2356 sh	
2322	(1)	2322 ms	$\nu_a(\text{ND}_3^+)$
2299 sh	(1)	2298 sh	
2251	(7)	2252 ms	$\left\{ \begin{array}{l} \nu_a(\text{CD}_3) \\ \nu_s(\text{ND}_3^+) \end{array} \right\}$
2235	(8)	2237 ms	
2200	(1)	2193 m	
2150	(3)		
2124	(1)		
2107	(2)		
2079	(3)	2085 m, br	$\nu_s(\text{CD}_3)$
		1971 mw	
		~1610 vs	$\nu_a(\text{COO}^-)$
1593	(2)	~1591 vs	
		1508 sh	
1444	(1)	1440 w	
1407	(4)	1409 s	$\nu_s(\text{COO}^-)$
1341	(10)	1343 s	$\delta(\text{C}^\alpha\text{H})$
1321	(1)	1320 w	
1289	(3)	1286 ms	$\delta'(\text{C}^\alpha\text{H})$
1192	(0)	1192 sh	$\delta_a(\text{ND}_3^+)$
1180	(1)	1180 m	
1150	(1)	1153 mw	$\delta_s(\text{ND}_3^+)$
1124	(2)	1126	ν (skeletal)
1077	(2)	1074 vw	$\delta_s(\text{CD}_3)$
1061	(3)	1062 sh	$\delta_a(\text{CD}_3)$
		1054 w	
1046	(0)	1045 mw	$\left\{ \begin{array}{l} \text{two } \nu \text{ (skeletal),} \\ \text{two } \rho \text{ (ND}_3\text{),} \\ \text{two } \rho \text{ (CD}_3\text{),} \\ \gamma \text{ (COO}^-) \end{array} \right\}$
1016	(9)	1016 vw	
		1011 sh	
968	(0)		
875 sh	(1)	873 mw	
867	(2)		
827	(2)	827 vw	
799	(6)	796 mw	
742	(7)	743 vw	
724	(1)	720 mw	
600	(3)	595 mw	$\delta(\text{COO}^-)$
505	(5)	509 ms	$\rho(\text{COO}^-)$
357	(0)	365 mw	$\delta(\text{C}^\beta\text{C}^\alpha\text{N})$
340	(3)	333 w	$\tau(\text{ND}_3^+)$
		326 w	
		322 vw	
		313 vw	
		300 vw	
285	(1)	287 sh	two δ (skeletal)
273	(1)	273 m	
259	(3)	251 mw	
		247 mw	
238	(2)		$\left\{ \begin{array}{l} \tau(\text{CD}_3), \tau(\text{COO}^-), \\ \text{and lattice modes} \end{array} \right\}$
205	(1)		
183	(2)		
157	(2)		
141 sh	(1)		lattice modes
128	(5)		
111	(26)		
103 sh	(3)		
96 sh	(5)		
71 sh	(3)		
62 sh	(3)		
47	(20)		

† See second footnote, Table 1.

Only two medium-to-strong bands lie between 1400 and 1250 cm^{-1} in the spectra of the methyl deuterated sample. We attribute these features at 1358 and 1299 cm^{-1} to the orthogonal pair of C^αH deformations. Therefore, previous assignments of the symmetric CH_3 deformation in the normal analog to a band at 1359 cm^{-1} [2, 3, 6, 7] are most probably erroneous. Instead, as shown in Fig. 3, the weak Raman band at 1378 cm^{-1} [3] must be assigned to this vibration, in agreement with well-known group frequency correlations for the $\text{CH}_3\text{-C}$ moiety [8, 9]. Earlier assignments were probably influenced by the absence of a band near 1378 cm^{-1} in the i.r. spectra of the undeuterated molecule. This feature is readily observable, however, in the i.r. spectrum of the N-deuterated species [4, 6].

In summary, the data obtained for methyl deuterated L-alanine lead to a revised set of assignments for the carbon-hydrogen stretching and deformation vibrations of normal L-alanine and its N-deuterated analog.

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